Hexapoda **17(1)**: 49-58, 2010 ISSN 0973-8592

LABORATORY EFFICACY OF *Lecanicillium lecanii* (ZIMMERMANN) AGAINST DIFFERENT STAGES OF *Helicoverpa armigera* AND ITS BIOSAFETY ON *Trichogramma* sp.

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ABSTRACT: The white halo fungus, *Lecanicillium lecanii* was evaluated for its insecticidal activity against larvae, pupae and adult stages of the American bollworm, *Helicoverpa armigera*. In general, the highest concentration of the fungus (2.4×10^7) led to least pupation. The length of larvae and pupae did not vary between the fungal treatments, while the weight showed a significant variation. Prolongation in larval and pupal duration and as a result, high percentage of malformed adults was noted. Subsequent effect on adults, particularly, the longevity and fecundity was reduced in the highest conc. The biosafety tests on *Trichogramma* sp. proved that even at the highest conc. the parasitization was on par with the untreated.

Key words: *Lecanicillium lecanii, Helicoverpa armigera, Trichogramma*

INTRODUCTION

American bollworm, *Helicoverpa armigera,* a polyphagous noctuid, feeds on 200 host plants in India, and is most damaging to cotton, chick pea, pigeon pea, tobacco, okra, sunflower, groundnut and sorghum (Pawar, 1998). Total losses due to *Helicoverpa* in cotton, vegetables and cereals may exceed 2 billion and the cost of insecticides used to control may be over $ 500 million annually (Sharma, 2001).

Of the many methods to manage pests, chemical insecticides have been in use for several decades. However repetitive use of synthetic chemicals for several decades to manage this pest resulted in serious health hazards, widespread ecological damage (Rejaul Hassan and Karim Bakshi, 2005) and development of resistance to a variety of insecticides (Kranthi *et al.,* 2002).

Hence, search for viable and sustainable alternatives to synthetic pesticides is given priority. Microbial pesticide is one such alternative which offers a new method to control insecticide resistant population of *H. armigera*, which would further prevent the emergence of secondary pest problems (Pawar and Borikar, 2005).

Entomopathogenic fungi are ubiquitous and are capable of natural recycling in appropriate hosts. Unlike bacteria, fungi cause direct penetration through the host cuticle without the requirement of ingestion (Pell *et al.,* 2001). Moreover, fungal pathogens particularly *Beauveria bassiana, Metarhizium anisopliae, Lecanicillium lecanii* and *Nomuraea rileyi* have been found to be promising in the control of several agricultural pests (Lingappa *et al.,* 2005) and in particular against *H. armigera* in different crops (Pawar and Borikar, 2005). They are also specific and safe to the environment (Saxena Hem, 2008).

The entomopathogenic fungus, *L. lecanii* naturally infects a wide range of sucking pests such as thrips, aphids and mites that are economically important pests of major horticultural crops (Kulkarni *et al.,* 2003), white flies, *Bemesia tabaci* (Meade and Byrne, 1991) and coffee green bug, *Coccus viridis* (Easwaramoorthy and Jayaraj, 1978). A critical literature survey reveals that *L. lecanii* has not been studied in an in-depth manner for its pesticidal character against the target pest. Hence, the present study aimed to explore the insecticidal activity of *L. lecanii* against *H. armigera*.

MATERIALS AND METHODS

***H. armigera*- source:** *H. armigera* larvae were collected from the fields of lady's finger, cotton and chickpea from Kannivadi in Dindigul district.

Mass rearing of *H. armigera*: The larvae collected from various hosts were maintained in the laboratory at 22 ± 2°C and 70 – 75 % RH. The larvae were reared on semi-synthetic diet (Shorey and Hale, 1965; Sathiah, 1987) in individual containers to prevent cannibalism and contamination.

Media used: Potato Dextrose Agar (PDA) (pH-5.6±0.2)-(Himedia)-39 g in 1000 ml distilled water.

Fungal source: *Lecanicillium lecanii* culture was obtained from National Bureau of Agriculturally Important Insects (NBAII) (Formerly PDBC), Bangalore, India.

BIOASSAY

Preparation of spore suspension: Spore suspension was prepared from 15 days old cultures of *L. lecanii* on PDA. The fungal surface was scraped using a sterile loop with 10 ml of sterile distilled water having 0.02% Tween 80 as a wetting agent (Rombach *et al.*, 1986). The suspension was then filtered through sterile muslin cloth to eliminate the medium (Sasidharan and Varma, 2005). Spore concentration of the filtrate was determined using a Neubauer hemocytometer. This served as the stock suspension. Spore suspension of *L. lecanii* at four different concentrations, 2.4×10^7, 2.4×10^6, 2.4×10^5 and 2.4×10^4 spores/ml was prepared by adding sterile 0.02% Tween 80 in distilled water and tested for the efficacy on different stages of *H. armigera*.

Growth inhibition of larvae (Hafez *et al.*, 1992; Kulkarni and Lingappa, 2002): Third instar larvae of *H. armigera* were treated by dipping method. Bengal gram pod was soaked in water overnight and then in the four different concentrations of the spore suspensions of *L. lecanii* and controls (sterile distilled water, 0.30% (v/v) of neem product and 0.006 % (v/v) of cypermethrin) for 30 minutes. It was then transferred into individual vials and the larva was allowed to feed on the treated pods. After the larvae had finished feeding on the gram pod, it was fed with semi-synthetic diet. Ten larvae were used in one replication. Each treatment was replicated thrice. Growth parameters namely larval duration (days), larval length (cm), larval weight (mg) and pupation (%) were recorded.

Growth inhibition of pupae (Hafez *et al.*, 1992): The pupa of the resultant progeny from the respective treatments, if any, was bioassayed. The four different spore concentrations of *L. lecanii* with three replications were used for infecting the pupa of *H. armigera*. The pupae were treated with 9 ml of the respective fungal spore suspensions using a hand atomizer. The pupae treated with sterile distilled water, neem product (0.30% v/v) and cypermethrin (0.006% v/v) served as the controls. The growth of surviving pupa was maintained up to adult emergence and the parameters such as pupal duration (days), pupal weight (mg), pupal length (cm) and adult emergence (%) was recorded.

Adult longevity, fecundity and egg hatchability (Malarvannan, 2004): Healthy adults were released into mud pots at 1:1 male-female ratios. Cotton swabs dipped in 10% honey treated with 1 ml of the test fungi served as treatment. The experiment was performed using four different spore concentrations of the test fungi, *L. lecanii* against *H. armigera*. Cypermethrin and Neem served as controls. Adult longevity (days), fecundity (numbers) and hatchability (%) were recorded. Triplicates were maintained for each treatment.

Biosafety of *L. lecanii* against egg parasitoid, *T. chilonis* and *T. japonicum* (Sharad and Sabir, 2005) Preparation of egg cards: Fresh *Corcyra cephalonica* eggs (12 h old) collected from the insectary was cleaned and taken in glass Petri dishes (15-20 cm diameter). Eggs were sterilized with UV light (156W) in a closed chamber for a half-an-hour duration, so that the embryo killed without damaging other egg contents. The UV sterilized eggs were sprinkled on thick cards (5 x 2 cm) smeared with a thin layer of diluted gum at 50 eggs/card. Four different concentrations of fungal spore suspensions and controls (neem and cypermethrin) were sprayed on the *C. cephalonica* eggs using manually operated atomizer. Untreated check was also maintained. Honey solution (10 %) was streaked on the smooth side with a camlin brush. This sheet was folded and stapled in such a way that honey surface is inside and the adults feed on the honey through the holes from the eruptive surface. The experiment was maintained at room temperature of 25 ± 2° C and at 60 % R.H.

Inoculation of parasitoids: The pre-conditioned and fungal treated *C. cephalonica* egg cards were dried under fan and placed inside the glass vials and a pair of *T. chilonis* and *T. japonicum* was segregated from the pool culture and introduced into the vial for parasitization. The adult wasps started parasitizing the *Corcyra* eggs. Three days after inoculation, daily observation for blackening of eggs was made till adult emergence to check the percent parasitization. Parasitoids under above mentioned

room temperature and relative humidity emerged on the 7th and 8th day. The number of progeny adults emerged, sex ratio, percent emergence of adult in each vial were assessed and recorded.

Statistical analysis: The data were statistically analyzed using AGRES package version 4 and SPSS version 9.

EXPERIMENTAL RESULTS

Efficacy of *L. lecanii* against different stages of *H. armigera:*

A) Larvae: The larvae treated with the four concentrations of *L. lecanii* were assessed for various developmental parameters such as pupation (%), larval length (cm), larval weight (mg), larval duration (days), pupal length (cm), pupal weight (mg), pupal duration (days) and percent moth emergence (healthy and malformed). Longevity, fecundity and egg hatchability of the resultant adults were also recorded.

Among the different fungal concentrations, the least pupation was noticed in 2.4×10^7 (53.3%) followed by 2.4×10^6 and 2.4×10^5 (60.0%). However the pupation was least in cypermethrin and neem product (13.3%) whereas in control 100% pupation was observed. In addition, the fungal growth was observed on the larvae (Plate 1d), which confirms the efficacy of the biocontrol agent. The larval mortality was observed on the 3-7 days after treatment.

The larval length was more or less similar in all the treatments, which ranged from 2.1 – 2.5 cm except for neem and cypermethrin (0.3 and 0.4 cm), whereas the length of healthy larvae was 2.9 cm (Table 1).

It was different in the case of larval weight. It was least (261.8 mg) in 2.4×10^4 treatment compared to control 389.8 mg (Table 1).

The larval duration was 5.4 days in 2.4×10^7 as against the untreated (12.6 days). As neem product influences the moulting process and insecticide has a tendency to exercise quick mortality and resulted in least pupation, the average larval duration was least in those treatments (1.8 and 2.0 days).

B) Pupa: The pupal weight ranged from 22.3 – 310.6 mg. Comparison between the different fungal treatments revealed that least pupal weight (154.9 mg) was recorded in 2.4×10^7. In other treatments, nearly 59% and above reduction was observed when compared to control. The results varied significantly at 5% level (Table 2).

There was variation in the pupal size. In general, the pupal size was reduced in many treatments (0.1 – 1.1 cm) compared to control (normal diet –1.8 cm). The trend was similar to pupal weight in which the least (0.9 cm) pupal length was observed in 2.4×10^7 (Plate 2a; Table 2).

Among the different treatments, healthy moth emergence was severely affected in larvae treated with neem (0%) followed by *L. lecanii* at 2.4×10^4 (11.1%) (Plate 3) and cypermethrin (16.6%). The highest percentage (93.3%) of healthy moth emergence was recorded in larvae maintained on normal diet (Table 2). Significant reduction in pupation with larval–pupal intermediates (due to phagodepression and difficulty in moulting) was observed (Tables 1-2 and Plate 2b).

C) Adult: In general, the longevity of adult varied from 0 to 12 days between treatments. With the honey solution the adults lived longer (12 days) except for neem in which the adults died immediately (Fig 1). In fungal treatments, an early adult mortality (0.0 days) was observed with 2.4×10^7, followed by cypermethrin (2.0 days).

In general, there was a wide variation in the fecundity among different treatments. Few treatments *viz.*, 2.4×10^7, cypermethrin and neem arrested the fecundity completely, which was followed by 2.4×10^6 (212 nos) (Fig 1).

The egg hatchability was suppressed in most of the treatments. The highest percentage 93.3 was recorded with normal control, where as it was nil in 2.4×10^7, cypermethrin and neem (Fig 1).

Biosafety of *L. lecanii* against egg parasitoids, *T. chilonis* and *T. japonicum*: The fungal suspensions at four concentration levels (2.4×10^4, 2.4×10^5, 2.4×10^6 and 2.4×10^7) were tested for its safety on *T. chilonis* and *T. japonicum*. The parameters observed were percent parasitization and wasp emergence (Numbers) (Table 3; Figure 2).

a) Parasitization: Among the different treatments tested, even at the highest concentration viz., 2.4×10^7 the parasitization by *T. chilonis* was 88.2% followed by 83.5% by *T. japonicum* which was on par with the control (91%) (Table 3). It is proved that entomopathogenic fungus could be quite specific and might infect only certain type of insect host. Least parasitization was observed in neem and cypermethrin (70.9 and 70.4%) (Table 3). Among the fungal treatments, parasitized but unhatched eggs in both *T. chilonis* and *T. japonicum* were more at 2.4×10^4 (18.7 and 21.2%). However, it was high in the case of neem and cypermethrin.

b) Adult emergence: The male female ratio in *T. chilonis* was more or less equal in the fungal treatments, which was on par with the untreated. This clearly shows that the adult emergence is not affected by the fungus. The females emerged was highest in 2.4×10^7 (16.3) as against least in neem (3.7). (Figure 2a). However, the male female ratio in *T. japonicum* was slightly irregular. The maximum number of female wasps was recorded in 2.4×10^4 (17.0), which were more than the untreated. This proves that fungus would not cause any adverse effect against the parasitoid (Figure 2b).

DISCUSSION

The results obtained for pathogenicity of *L. lecanii* against *H. armigera* larvae (Table 1), showed that it is efficient. From the results, it was clear that the pupation (%) decreases with increase in spore concentration of *L. lecanii*. It may be due to the loss in apatite which would have been due to the fungus. With entomophthoralean fungi, unicellular yeast-like cells with chitinous walls (hyphal bodies) spread throughout the insect obtaining nutrients, leading to the death of the host by physiological starvation about 3-7 days after infection (Shah and Pell, 2003). This suppressive effect may be due to the inhibitory action on mitochondrial respiration by affecting the NADH- Cytochrome C-reductase and complex-I of insect mitochondria (Londershausen *et al.*, 1991).

Forced early adult emergence was observed in those treated with *L. lecanii*. The pupal duration was 1.6 days in 2.4×10^4 as against the untreated (10.2 days). However, in this case, the pupal duration was on par with neem and cypermethrin. In contrast, the pupal duration was prolonged in *Beauveria bassiana* treated pupae of *Phthorimaea operculella* compared with the control (Hafez *et al.*, 1994).

The decrease in the juvenile hormone titre and its associated disturbances in oogenesis, larval-pupal and pupal-adult moults are interpreted as an interference with moulting hormone pools (Rembold *et al.*, 1982). Decrease in juvenile hormone influences the storage proteins and fat body, which are highly essential for metamorphosis, moulting and reproduction (Palli and Locke, 1987; Koul and Isman, 1991).

In fungal treatments, an early adult mortality (0.0 days) was observed with 2.4×10^7, followed by cypermethrin (2.0 days) (Fig 1). The longevity of adult males of *Phthorimaea operculella* was reduced to 9.3 days at 16.5×10^8 conidia/ml of *B. bassiana* as compared to 12.9 days in the control (Hafez *et al.*, 1994).

Few treatments *viz.*, 2.4×10^7, cypermethrin and neem arrested the fecundity completely, which was followed by 2.4×10^6 (212 nos) (Fig 1). The egg hatchability was nil in 2.4×10^7, cypermethrin and neem (Fig 1). The red palm weevil adults, *Rhynchophorus ferrugineus* when treated with *M. anisopliae* and *B. bassiana* increased egg mortality and reduced their hatchability. The total percentage mortality of eggs and hatched larvae was 80-82% (Gindin *et al.*, 2006). Khodadad *et al.* (2006) reported similar results of remarkable effects of *M. anisopliae, B. bassiana* and *Lcanicillium psalliotae* on the egg hatchability (%) and reproductive efficiency of *Rhipicephalus* (*Boophilus*) *annulatus*.

Among the different treatments tested, even at the highest concentration viz., 2.4×10^7 the parasitization by *T. chilonis* was 88.2% followed by 83.5% by *T. japonicum* which was on par with the control (91%). The results were supported by Broza et al. (2001) and Dromph and Vestergaard (2002) who noted that *B. bassiana, B. brongniartii, Hirsutella* spp, *M. anisopliae and V. lecanii* did not affect the mortalities of three Collembolan species. Previously, James and Linghthart (1994) reported that *M. anisopliae* did not have the potential to infect *Hippodamia convergens.* In contrast, *Photorhabdus luminescens,* a symbiotic bacterium associated with the entomopathogenic nematode, *Heterorhabditis indica* caused more than 84% mortality of the *Trichogramma* (Sharad and Sabir, 2005).

According to Greathead and Prior (1990) there have been no signs of intolerance adverse effects by *Metarhizium flavoviride* on non-target species. However Goettel *et al.* (1990) postulated that side effects can be expected in a wide range of non-target arthropods. Peveling and Demba (1997) tested blastospores of *M. flavoviride* on *Pharoscymnus anchorago* L. (Coleoptera: Coccinellidae) and recorded no adverse effects of the entomopathogens on this West African lady bird beetle. However, Ball *et al.* (1994) carried out laboratory experiments with *M. flavoviride* on *Apis mellifera* and found that bees can be infected if kept under stress.

ACKNOWLEDGEMENTS

The authors acknowledge Dorabji Tata Trust, Mumbai for the financial support.

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Table 1. Effect of *L. lecanii* on growth of *H. armigera* larvae

Treatment		Characters: Larval growth — Larval length (cm)	Larval weight (mg)	'Larval duration (days)	Pupation (%)
Control	Untreated	2.9[b]	389.8[bc]	12.6[c]	100.0[c]
	Cypermethrin 0.06% (v/v)	0.4[a]	25.4[a]	2.0[a]	13.3[a]
	Neem 0.30% (v/v)	0.3[a]	26.5[a]	1.8[a]	13.3[a]
	2.4 x 10^4	2.1[b]	261.8[b]	7.4[b]	66.6[b]
L. lecanii (spores/ml)	2.4 x10^5	2.2[b]	304.2[bc]	6.1[b]	60.0[b]
	2.4 x 10^6	2.5[b]	420.5[c]	6.0[b]	60.0[b]
	2.4 x 10^7	2.3[b]	296.6[bc]	5.4[b]	53.3[b]
	CD (P=0.05)	1.2	157.2	3.1	24.1

Each value mean of triplicate

Different letters in each column differ significantly (5%) by LSD

Table 2. Effect of *L. lecanii* on growth of *H. armigera* pupae

Treatment		Characters				
		Pupal growth			Adult emergence	
		Pupal Weight (mg)	Pupal Length (cm)	Pupal Duration (days)	Healthy (%)	Malformed / Dead Pupa (%)
Control	Untreated	310.6^{c}	1.8^{c}	10.2^{b}	93.3^{b}	6.6^{a}
	Cypermethrin 0.06% (v/v)	22.3^{a}	0.1^{a}	2.0^{a}	16.6^{a}	83.3^{b}
	Neem 0.30% (v/v)	27.1^{a}	0.2^{a}	0.0^{a}	0.0^{a}	100.0^{b}
L. lecanii (spores/ml)	2.4 x 10^{4}	185.3^{b}	1.1^{b}	1.6^{a}	11.1^{a}	88.9^{b}
	2.4 x10^{5}	182.2^{b}	1.0^{b}	2.8^{a}	22.3^{a}	77.7^{b}
	2.4 x 10^{6}	187.5^{b}	1.0^{b}	2.6^{a}	22.3^{a}	77.7^{b}
	2.4 x 10^{7}	154.9^{b}	0.9^{b}	2.7^{a}	22.2^{a}	77.8^{b}
CD (P=0.05)		70.0	0.3	5.1	39.4	39.4

Each value mean of triplicate
Different letters in each column differ significantly (5%) by LSD

Table 3. Biosafety of *L. lecanii* to egg parasitoid, *Trichogramma* sp.

Treatment		Parasitization (%)			
		Parasitized but unhatched	Parasitized and hatched	Parasitized but unhatched	Parasitized and hatched
		Trichogramma chilonis		*Trichogramma japonicum*	
Control	Untreated	8.8^{b}	91.2^{a}	8.8^{a}	91.2^{a}
	Neem plus 0.30%(v/v)	29.0^{a}	70.9^{b}	29.0^{b}	70.9^{b}
	Cypermethrin 0.006%(v/v)	29.5^{a}	70.4^{b}	29.5^{b}	70.4^{b}
L. lecanii (spores/ml)	2.4 × 10^{4}	18.7^{ab}	81.3^{ab}	21.2^{ab}	78.7^{ab}
	2.4 × 10^{5}	11.9^{b}	88.0^{a}	18.1^{ab}	81.8^{ab}
	2.4 × 10^{6}	15.5^{b}	84.4^{a}	20.3^{ab}	79.6^{ab}
	2.4 × 10^{7}	11.8^{b}	88.2^{a}	16.4^{a}	83.5^{a}
CD (P=0.05)		13.2^{b}	13.2	12.4	12.4

Each value mean of triplicate
Different letters in each column differ significantly (5%) by LSD

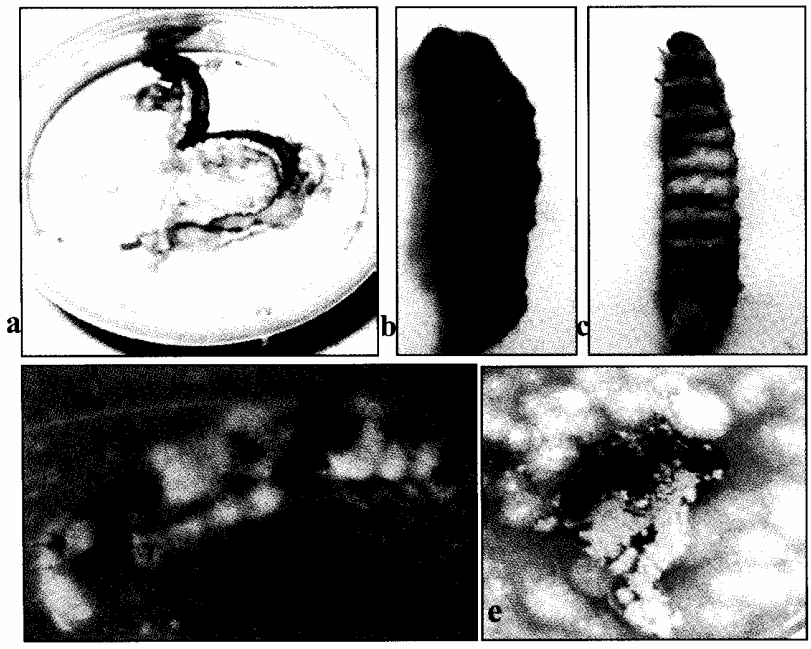


Plate 1

Plate 1. Effect of *L. lecanii* on the growth and development of *H. armigera*
a- lethargic larva; b & c – larval-pupal intermediate; d-dead cadaver;
e- fungus growth from the cadaver fluid

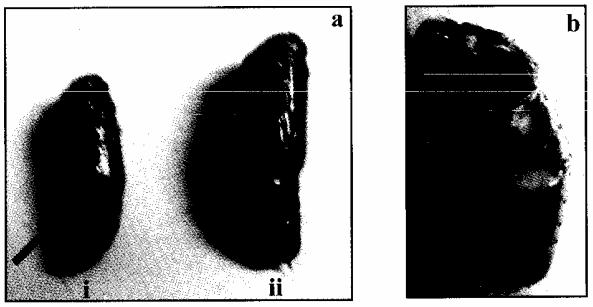


Plate 2
Juvenomimetic activity of *L. lecanii* against *H. armigera*
a. Pupal weight reduced by *L. lecanii* – i – fungal treatment; ii – control;
b- incomplete metamorphosis

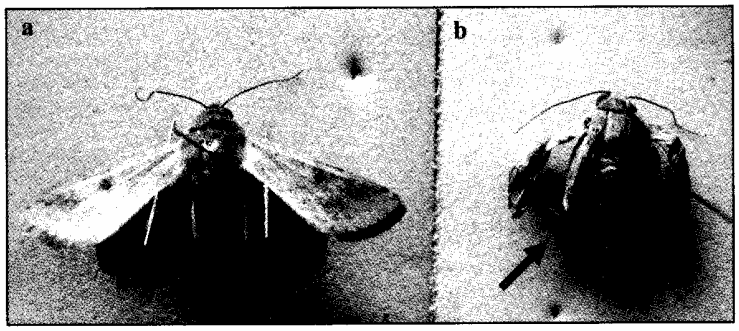
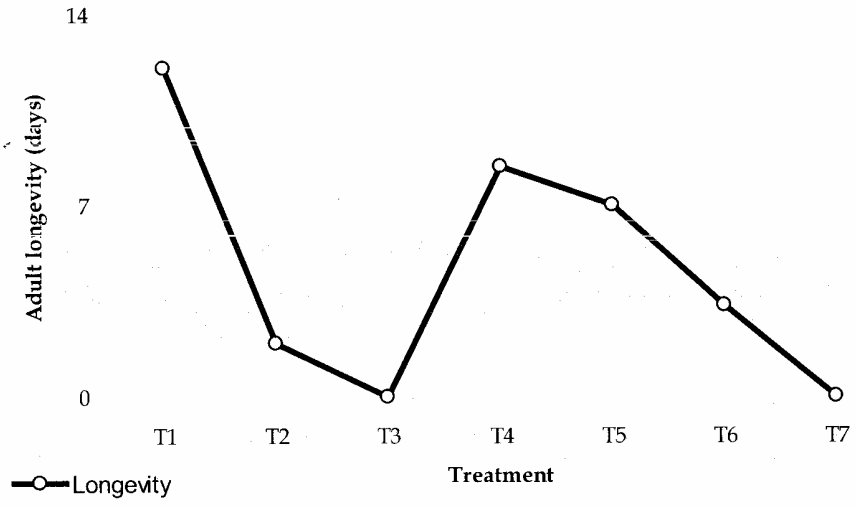
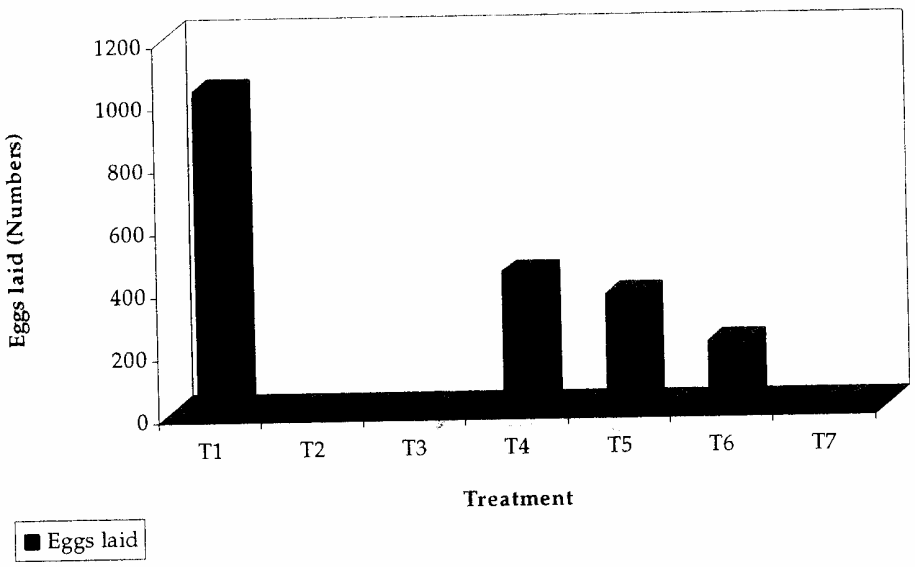


Plate 3
Effect of *L. lecanii* on *H. armigera* adult emergence
a-Healthy adult; b- malformed adult – deformed wings (arrow)





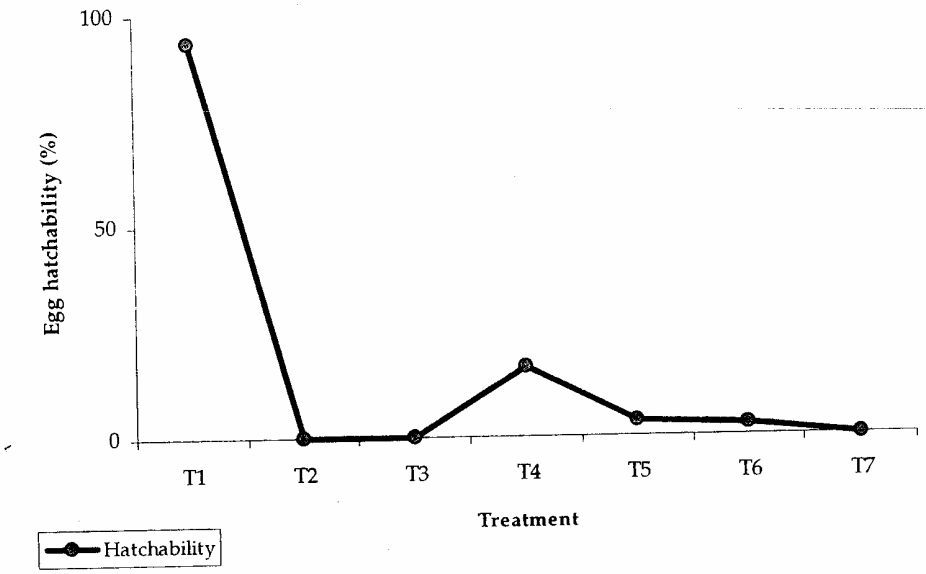


Fig. 1. Effect of *L. lecanii* on longevity, fecundity and hatchbility of *H. armigera*
T1- Control (10% honey solution); T2- cypermethrin; T3- neem plus;
T4- 2.4×10^{4}**;T5 –** 2.4×10^{5}**;T6-** 2.4×10^{6}**; T7-** 2.4×10^{7}

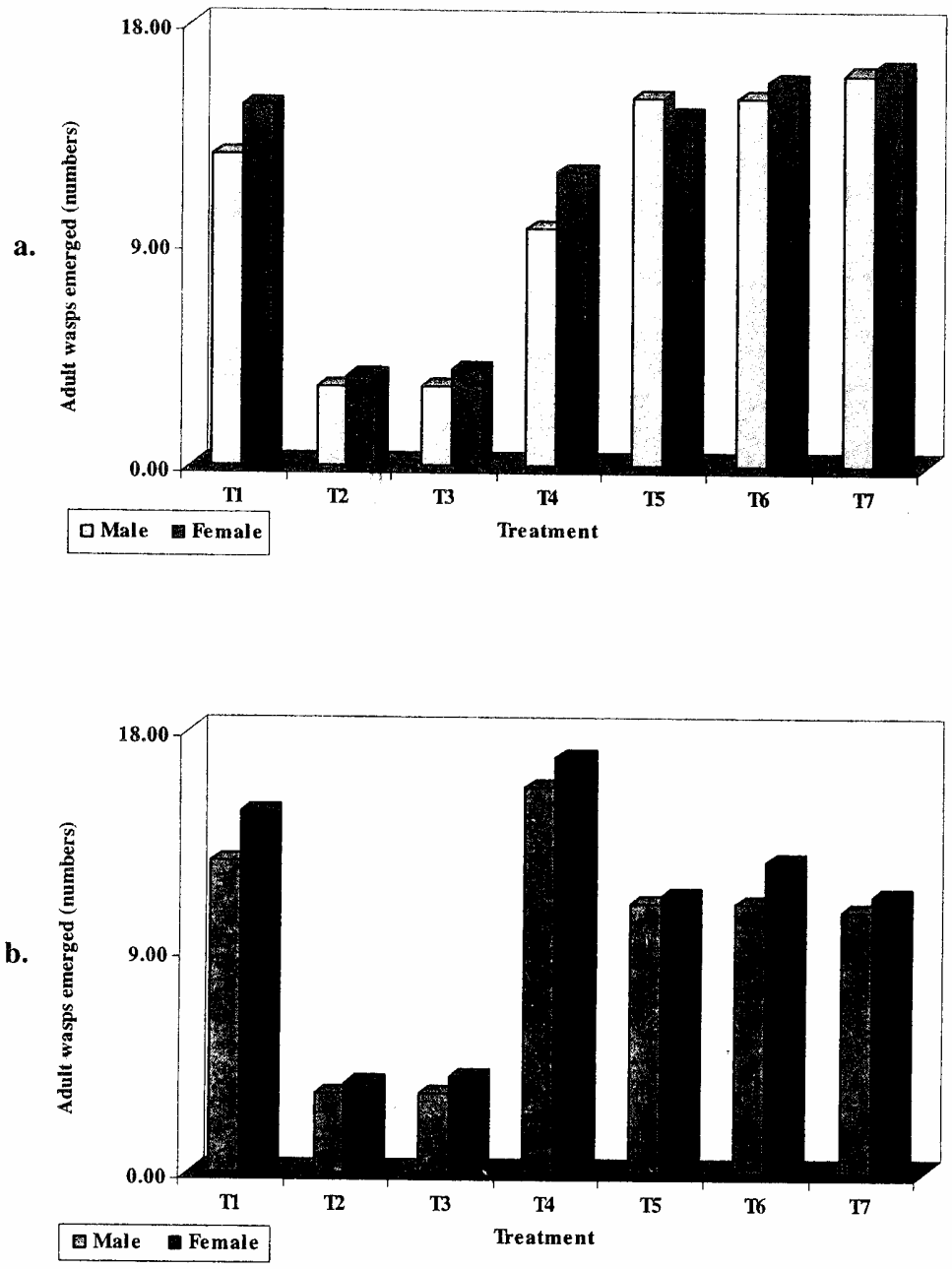


Fig. 2. Biosafety of *L. lecanii* to egg parasitoids, *Trichogramma* spp.
T1- Untreated; T2 – Neem plus; T3 – Cypermethrin;
T4- 2.4×10^4**;T5 –** 2.4×10^5**;T6-** 2.4×10^6**; T7-** 2.4×10^7
a - Trichogramma chilonis;* b – *Trichogramma japonicum